Attorney Docket No.: 10094.210-US PATENT

**IN THE UNITED STATES PATENT AND TRADEMARK OFFICE**

In re Application of: Sjøholm et al. Confirmation No: 9916

Serial No.: 10/713,394 Group Art Unit: 1656

Filed: November 14, 2003 Examiner: M. Monshipouri

For: Use of Acid Stable Protease in Animal Feed

**SECOND DECLARATION UNDER 37 CFR 1.132**

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Sir:

I, Anna-Maria Klünter, do hereby state and declare that

1. I am the same Anna-Maria Klünter who submitted a Declaration under 37 CFR 1.132 in U.S. application no. 09/779,323, which is the parent of the above-identified application ("First Declaration"). A copy of my First Declaration was previously submitted during the prosecution of the above-identified application. As I mentioned in my First Declaration, I received a doctoral degree in Agricultural Sciences from the Agricultural Faculty of the Rheinische Friedrich-Wilhelms-University in Bonn, Germany. From 1988 to 1990 I had a post-doctoral position in Animal Nutrition Research of F. Hoffmann-La-Roche Ltd., Switzerland. Since 1990, I have been employed by Société Chimique Roche SA (since 2001 Roche Vitamines France S.A.S., since 2004 DSM Nutritional Products France SAS), France. From 1990 – 1994, I was a Research Scientist, and from 1994 until 2003, I have been the Group Leader for the research group Poultry Nutrition. From 2003 until 2004, I have been Section Head of the Animal Nutrition Research. Since 2004 I am Research Center Manager Animal Nutrition & Health. During the last fifteen years, my responsibilities have involved developing different enzymes for animal feed.

2. The U.S. Patent and Trademark Office has rejected claims 27-35 and 38-39 of the above-identified application under 35 U.S.C. 103 as being unpatentable in view of Bedford et al. (WO 96/05739) in view of JP 02255081 (Snow-Brand Milk Prod.). Claims 27-35 and 38-39 are drawn to methods for improving the nutritional value of an animal feed or a vegetable

protein, comprising adding to the animal feed or the vegetable protein an acid-stable protease comprising an amino acid sequence having an identity of at least 90% to SEQ ID NO: 1. The Office stated that my First Declaration does not support the entire scope of the claims. I respectfully disagree that the combination of Bedford et al. and JP 02255081 renders these inventions obvious.

3. JP 02255081 discloses a protease produced by *Nocardiopsis* sp. OPC-210 (FERM P-10-508). However, JP 02255081 does not teach or suggest either animal feed additives and compositions comprising, or methods for improving the nutritional value of an animal feed, using a protease which comprises the amino acid sequence of SEQ ID NO: 1.

4. Bedford et al. disclose the use of various enzymes, including proteases, in animal feed compositions. As explained in my First Declaration, all of the proteases described in Bedford et al. are alkaline proteases and not acid-stable proteases and the results disclosed in Bedford et al. do not demonstrate that the addition of a protease to an animal feed results in an improved feed conversion ratio or that the final body weight was significantly increased using any of the animal feeds comprising a protease.

5. As also explained in my First Declaration, broiler chickens have a significantly improved weight gain and significantly improved feed conversion when fed an animal feed composition comprising the protease of SEQ ID NO: 1 (*Nocardiopsis sp.* NRRL 18262 protease). These results are surprising and unexpected.

6. Furthermore, persons skilled in the art would expect that proteases having an amino acid sequence which is highly homologous, *e.g.*, at least 90% identity, to the sequence of SEQ ID NO: 1 would also result in an improved nutritional value of the feed. This is supported by the following experiments, which was carried out under my supervision.

7. 240 chickens per treatment were sorted at day 8 into twelve replicate groups of twenty chickens (six groups of males and six groups of females) and were fed for fourteen days (days 8 – 22) with the animal feed composition shown in table 1 below:

**Table 1:** Feed composition of the experimental diet

| | |
|---|---|
| Ingredients (%): | |
| Maize | 20.00 |
| Maize starch | 26.00 |
| SBM 44 | 44.20 |
| Soybean oil | 4.50 |
| DL-Methionine | 0.30 |
| MCP | 1.60 |
| Limestone | 1.20 |
| Salt | 0.10 |
| Binder | 1.00 |
| $TiO_2$ | 0.10 |
| Premix [1] | 1.00 |
| Calculated content: | |
| Crude protein (%) | 19.9 |
| $ME_N$ (MJ/kg) [2] | 12.5 |
| Crude fat (%) | 7.0 |
| Lysine (%) | 1.18 |
| Methionine (%) | 0.59 |
| Methionine + Cystine (%) | 0.90 |
| Analyzed content: | |
| Crude protein (%) | 21.7 |
| $ME_N$ (MJ/kg) [3] | 12.4 |
| Crude fat (%) | 7.0 |

[1] Including Avatec
[2] Calculated with EC-equation
[3] Calculated with EC-equation based on analyzed nutrient content

The feed composition was treated with different amounts of a protease from *Nocardiopsis* DSM 43235 having an amino acid sequence of amino acids 1-188 of SEQ ID NO: 2 disclosed in WO 2004/111220, or with plain water as a negative control. This sequence of amino acids 1-188 of SEQ ID NO: 2 is about 83.5% identical to the sequence of SEQ ID NO: 1 (*Nocardiopsis sp.* NRRL 18262 protease) disclosed in the above-identified application. The protease in solid form was dissolved in 600 ml of water (450 kg feed per treatment for days 8-22) and sprayed onto the feed pellets. The pellets for the negative control treatment were sprayed with the same amount of pure water.

In the control treatment the chickens were fed the above-identified feed composition without protease, and in the other treatments the chickens were fed the above-identified composition with the protease in different concentrations as follows (mg enzyme protein per kg feed): 12.5 mg/kg and 25.0 mg/kg.

8. The results of the experiments are shown in Table 2, wherein the results of both sexes have been pooled because no significant interaction between treatment and sex occurred. In the table the Newman-Keuls test has been applied to the results to indicate the statistical significance, hence the mean values within a row not sharing a common superscript are significantly different ($p < 0.05$).

These results demonstrate that broiler chickens have a statistically significant improved feed conversion when fed an animal feed composition comprising the *Nocardiopsis* DSM 43235 protease. These results demonstrate that proteases having an amino acid sequence which is highly homologous, *e.g.*, at least 90% identity, to the sequence of SEQ ID NO: 1 of the instant application would also result in an improved nutritional value of the feed.

**Table 2:** Performance of broiler chickens (day 8 – day 22)
Pooled results of both sexes; mean ± stdev

| | Control | Protease | |
|---|---|---|---|
| Dose (mg EP/kg) | 0 | 12.5 | 25.0 |
| Cages x birds | 12 x 6 | 12 x 6 | 12 x 6 |
| Weight gain (g/bird) | 757 $^{A}$ | 791 $^{A}$ | 793 $^{A}$ |
| Days 8-22 | ± 88 | ± 38 | ± 64 |
| % | 100.0 | 104.6 | 104.8 |
| Feed intake (g/bird) | 1100 $^{A}$ | 1125 $^{A}$ | 1122 $^{A}$ |
| Days 8-22 | ± 120 | ± 65 | ± 84 |
| % | 100.0 | 102.3 | 102.0 |
| Feed conversion (g feed/g gain) | 1.456 $^{A}$ | 1.422 $^{B}$ | 1.415 $^{B}$ |
| Days 8-22 | ± 0.040 | ± 0.049 | ± 0.026 |
| % | 100.0 | 97.7 | 97.2 |
| | | | |
| Mortality (%) | 2.6 | 1.4 | 1.4 |

Newman-Keuls test: means within a row, not sharing a common superscript, are significantly different ($p<0.05$)

9. I further declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further, that the statements are made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United

States Code, and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

Signed this 28 day
of April 2009

Anna-Maria Klünter